concept

The emergence of shape: notions from the study of the *Drosophila* tracheal system

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The generation of bodies and body parts with specific shapes and sizes has been a longstanding issue in biology. Morphogenesis in general and organogenesis in particular are complex events that involve global changes in cell populations in terms of their proliferation, migration, differentiation and shape. Recent studies have begun to address how these synchronized changes are controlled by the genes that specify cell fate and by the ability of cells to respond to extracellular cues. In particular, a notable shift in this research has occurred owing to the ability to address these issues in the context of the whole organism. For such studies, the *Drosophila* tracheal system has proven to be a particularly appropriate model. Here, my aim is to highlight some ideas that have arisen through our studies, and those from other groups, of *Drosophila* tracheal development. Rather than providing an objective review of the features of tracheal development, I intend to discuss some selected notions that I think are relevant to the question of shape generation.

Introduction

The larval tracheal system of *Drosophila* is a complex tubular network that conducts oxygen from the exterior to the internal tissues (Fig 1). Its development has been extensively reviewed (see, for example, Manning & Krasnow, 1993; Affolter & Shilo 2000; Affolter *et al*, 2003; Ghabrial *et al*, 2003) and can be briefly summarized as follows. The tracheal system arises from the tracheal placodes—clusters of ectodermal cells that appear at each side of 10 embryonic segments—extending from the second thoracic segment to the eighth abdominal segment. The cells of each cluster invaginate and migrate in a stereotypical pattern to form each of the primary tracheal branches. The general conclusion from many studies is that the direction of migration of the tracheal cells relies on a set of positional cues provided by nearby cells. In particular, all tracheal cells express the fibroblast growth factor (FGF) receptor Btl (Klambt *et al*, 1992), allowing them to respond to the FGF homologue Bnl,

which is expressed in clusters of cells surrounding the developing tracheal system at each position where a new branch will form and subsequently extend (Sutherland *et al*, 1996). A distinctive feature of this system is that once the tracheal cells invaginate, there is no further cell division. Therefore, the final acquisition of the shape and size of the tracheal system occurs without cell proliferation and the associated mechanisms, such as oriented cell division or uneven cell allocation following cell division.

Shape at interfaces

Shape often arises from the apposition between cell populations with different properties. This feature is illustrated by the initial steps of tracheal morphogenesis when cells invaginate to give rise to the tracheal pits. The formation of such an invagination requires some cells to move into the embryo while others remain at the embryonic surface. It is the confrontation between the cells moving inwards and those remaining in their external location that is responsible for the overall shape of the tracheal pit. The precise boundary between the cells that are selected to invaginate and those that are not is achieved by the restricted expression in discrete clusters of *tracheless* (*trh*) and *ventral veinless* (*vvl*), both of which encode transcription factors (Fig 2A,B). In mutant combinations leading to the ectopic expression of these two genes in a longitudinal stripe along the embryo, a longitudinal stripe of invaginating cells occurs, and the final shape of the new structure is determined by the newly generated interfaces between the cells that express these genes and those that do not (Llimargas & Casanova, 1997; Franch-Marro *et al*, 2006).

In the tracheal placode, invagination always begins at the same position, which is also defined by an interface: the border between dorsal cells that express the spalt (sal) transcription factor and ventral cells that do not. In addition, this interface not only determines where tube formation will arise but is also responsible for its final shape. On the dorsal side, *sal*-positive cells begin a rotation-like movement, folding to form a new layer of cells below the epidermal surface; by contrast, on the ventral side, cells slide below the invaginating dorsal cells (Fig 2C). As a result, the initial tube shape originates in a process that evolves a cell monolayer into a three-layer organization. In *sal* mutants, the dorsal cells do not rotate and the initial shape is not generated (Brodu & Casanova, 2006). It is still not clear how the juxtaposition between *sal*-positive and *sal*-negative cells is translated into the different cytological behaviours

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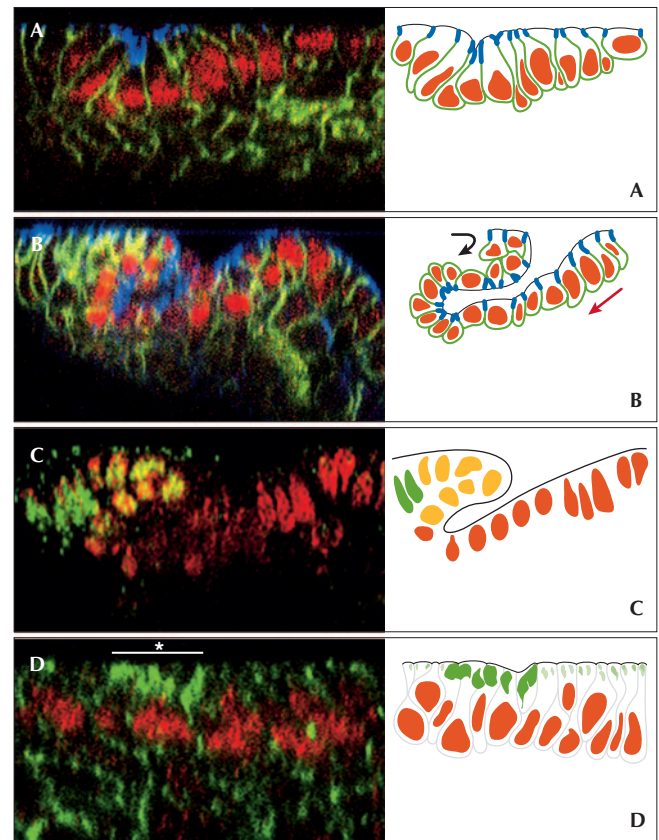Tel: +34 93 4034967; Fax: +34 93 4034979;
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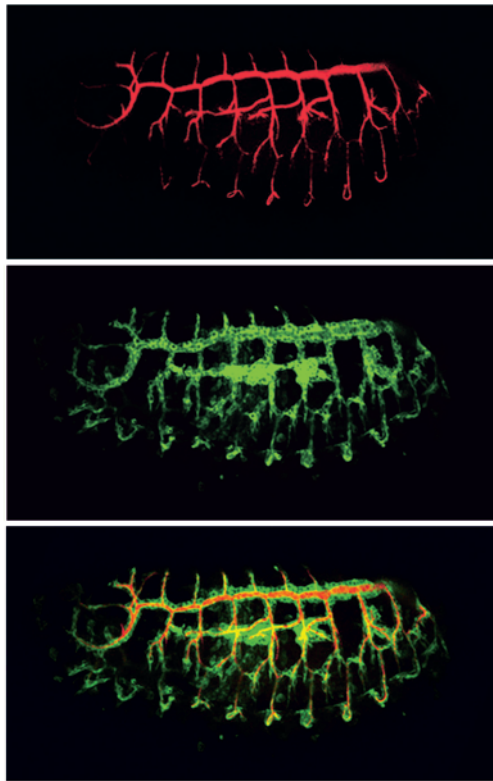
Fig 1 | The tracheal system of the *Drosophila* embryo. The embryonic *Drosophila* tracheal system as visualized with the 2A12 antibody (top), which recognizes a lumen component, and with a GFP antibody (middle) in an embryo carrying a UAS–tau-GFP reporter construct under the control of the tracheal-specific btl–GAL4 driver. A merged image is also shown (bottom). The tracheal system consists of different branches on the antero-posterior and dorso-ventral axes, each with a stereotypical pattern and size. Anterior is on the left and dorsal is at the top. Images provided by S. Araujo. GFP, green fluorescent protein.

Fig 2 | Interfaces and forces that shape body parts. (**A,B**) The tracheal cells (nuclei are shown in red) are selected to invaginate, whereas the neighbouring ectodermal cells remain at the embryonic surface. Tracheal cells are labelled using a Trh antibody. Anti-neurotactin labels the basolateral and basal sides of all epithelial cells (shown in green), whereas protein kinase C (PKC) labels their apical side (shown in blue). The arrows in (**B**) indicate the direction of movement. (**C**) Among the invaginating tracheal cells, the dorsal cells that express the gene *sal* begin a rotation-like movement, whereas the ventral cells slide below the dorsal ones. The tracheal cells are labelled as described in (**A**) and are shown in red, whereas the *sal*-positive cells are detected with a specific antibody (shown in green); tracheal *sal*-positive cells are visualized in yellow. (**D**) Accumulation of myosin II is detected at the onset of invagination in the cells initiating apical constriction (shown by the white line and asterisk), as visualized by a green fluorescent protein-tagged form of the myosin II light chain. Tracheal cells are labelled as described in (**A**) and are shown in red. Figures modified with permission from Brodu & Casanova, 2006.

that account for the emergence of a tubular shape. One possibility is that different genetic specifications could lead to cells with different forces or stiffness, and that the resulting apposition of two invaginating cell populations with such properties could force one of them to fold and initiate dorsal-oriented rotation, whereas the other slides under the former. Irrespective of the actual mechanisms, these examples clearly illustrate the emergence of shape at the interface between cell populations with different properties.

Shape by restriction: the power of constraint
Shape is often considered to result from a driving force—either internal or external—that acts as a positive patterning mechanism. However, analysis of the developing trachea in the context of the whole embryo shows that some of its features are clearly the result of constraints provided by the neighbouring tissues or organs. For example, cells from the tracheal cluster that will form the dorsal branches move dorsally along a groove between somatic muscle precursors from adjacent metameres (Fig 3A,B). The dorsal branches therefore end up positioned in the available space between the muscle precursor clusters, whereas no branches are

found inside the clusters (Franch-Marro & Casanova, 2000). Accordingly, redirection of branch outgrowth by ectopic expression of the Bnl chemoattractant is limited, and only happens at particular positions and paths (Sutherland *et al*, 1996). Besides this type of topological constraint, other mechanisms also restrict tracheal outgrowth to certain paths and therefore define by exclusion the shape of the tracheal system. Such is the case for the ganglionic branches, which are prevented from crossing the cells of the midline at the central nervous system (CNS). In this example, crossing is inhibited by expression of the molecule Slit in the

midline, which acts as a repellent for these tracheal branches in a manner similar to its role in axon guidance (Englund *et al*, 2002). Topological or signal-induced barriers can therefore act as constraints that contribute to the definition of organ shape by eliminating alternative arrangements.

Shape by cast: building on neighbours

Neighbouring cells not only constrain tracheal development; instead, subsets of surrounding cells also seem to act as a cast that moulds the precise shape of some branches. The shape of many of the primary tracheal branches therefore matches the distribution of specific subsets of mesodermal cells. The shapes of the dorsal trunk, lateral trunk and visceral branch depend, respectively, on their interactions with the lateral mesoderm, the lateral and ventral muscle precursors, and the visceral mesoderm (Fig 3C,D; Franch-Marro & Casanova 2000). Similarly, the shape of the ganglionic branches depends on their interactions with different subsets of neural and glia cells in the CNS (Englund *et al*, 1999). In a few cases, it has been possible to unravel some of the molecular mechanisms underlying these cell–cell interactions. For example, migration of the visceral branches requires the position-specific 1 (PS1) integrin in the migrating cells of the visceral branches and the complementary expression of the PS2 integrin in the visceral mesoderm substrate cells (Boube *et al*, 2001). Therefore, expression of given cell-surface molecules in a subset of tracheal cells, and in their neighbours, can ultimately determine the shape adopted by a particular tracheal branch. In the cases described here, shape is generated, at least in part, by moulding cells into a pre-existing shape that is determined by other groups of cells.

Shape by regulation of cell adhesion

Although the shape of the tracheal system requires tracheal cells to move as a distinct group and to change their position relative to surrounding cells, it also requires them to change positions among themselves. Tracheal cells therefore initiate movements as a cluster and maintain this adhesion as branches outgrow; however, as development proceeds, they can be allocated to different branches or can even change their relative positions within branches. This is conspicuous in the case of cell intercalation, during which tracheal cells in some branches evolve from being in a side-by-side position to a one-cell row (Fig 3A,B). During this process, intercellular junctions between tracheal cells change to autocellular junctions (Jazwinska *et al*, 2003). These cell rearrangements are responsible for the final shape of the tracheal branches and, although they are regulated genetically (Ribeiro *et al*, 2004), it is still not known how these rearrangements are accomplished. Tracheal morphogenesis therefore requires tracheal cells to develop a particular adhesion between one another, but, conversely, their adhesion must also be flexible enough to allow their controlled rearrangement at different developmental stages. Hence, it is not surprising to find that the expression of genes coding for different junctional proteins seems to be specifically regulated in the tracheal cells (see, for example, Baumgartner *et al*, 1996; Tanaka-Matakatsu *et al*, 1996; Llimargas *et al*, 2004; Jung *et al*, 2006). Furthermore, it has been found recently that epidermal growth factor receptor (EGFR) signalling modulates DE-cadherin accumulation at the cell membranes to regulate the adhesion between tracheal cells during their migration and to ensure the integrity of the tracheal system and its final shape (Cela & Llimargas, 2006). Together, these results suggest the
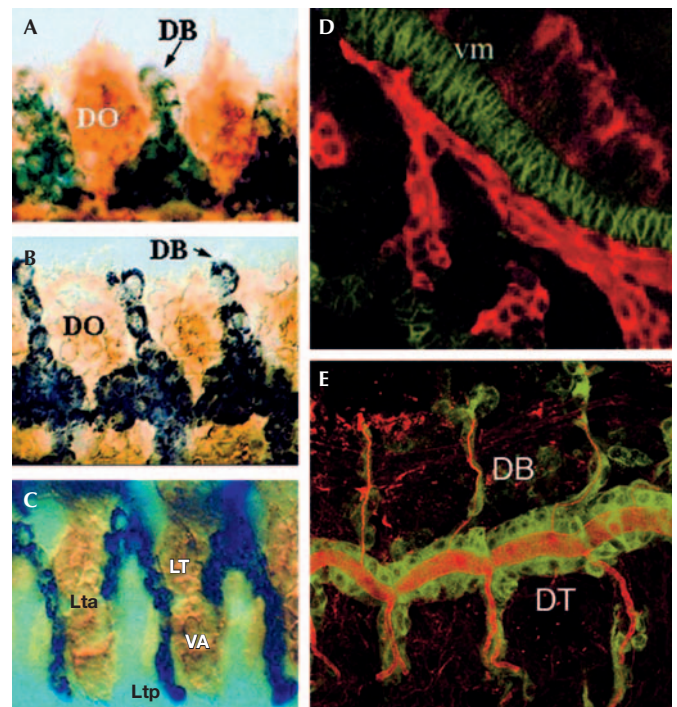
Fig 3 | Constraints, cast and size. (**A,B**) The tracheal cells of the dorsal branches (DB; shown in black) are located between the precursors of the dorsal oblique (DO) muscles (shown in brown). Note that by stage 14, one can observe several tracheal cells in the same row (**A**), whereas by stage 15, on intercalation, they form a one-cell row (**B**). (**C**) The tracheal cells of the lateral trunk anterior and posterior (Lta and Ltp, respectively; shown in black) form two branches in apposition to the lateral transverse (LT) and ventral acute (VA) muscles (shown in brown). Note that the shapes of Lta and Ltp result from their close apposition to the mesodermal cells. (**D**) The shape of the visceral branch results from the apposition of the tracheal cells (shown in red) to the cells of the visceral mesoderm (vm; shown in green). (**E**) In the dorsal trunk (DT; shown in the antero-posterior axis) two or more cells contribute to the tube circumference. Conversely, in the DB (shown in the dorso-ventral axis), the tube circumference is made from single cells wrapped around the lumen. In (**A–C**), mesoderm cells are visualized in embryos carrying the *twi-CD2* gene stained with an antibody against CD2, and the tracheal cells are visualized with the antibody against Trunk (Trk) in embryos carrying tracheal-specific btl–GAL4 and UAS–trk constructs. Images modified with permission from Franch-Marro & Casanova (2000). In (**D**), tracheal cells are visualized with a β-galactosidase antibody in embryos from the 1-eve-1 enhancer trap line, and the visceral mesoderm is detected by a FasIII antibody. Image modified with permission from Boube *et al* (2001). In (**E**), tracheal cells are detected by tracheal expression of a tau-GFP construct and the tracheal lumen by wheat germ agglutinin (Araujo *et al*, 2005). GFP, green fluorescent protein.

control of cell adhesion and the regulated transition between different degrees in its stiffness or plasticity as a mechanism behind the generation of specific shapes.

Forces that shape

Tracheal shape arises from forces that drive changes in cell morphology and movements. As mentioned above, tracheal branches

outgrow towards clusters of neighbouring cells that express *bnl*; this signal is received by the tracheal cells and prompts their migration. However, we still do not know how this signal is able to generate the force that drives cell movements. Similarly, the same pulling force generated by the leading cells towards the Bnl clusters has been postulated to trigger the process of cell intercalation (Ribeiro *et al*, 2004). In these cases, an external signal—once it has been transduced into the cell—triggers an unknown mechanism that generates the force that shapes the tracheal branches. Conversely, in other steps of tracheal morphogenesis, such as tracheal invagination, the driving force of cell rearrangements seems to be triggered by a signal from the tracheal cells themselves: the expression of *trh* in tracheal cells activates EGFR signalling, which, in turn, leads to the spatial and temporal organization of tracheal invagination. In this case, we know that the mechanism activated to drive tracheal invagination implies the apical localization of myosin II, which has an established role as an actin-based motor providing contractile force (Fig 2D; Brodu & Casanova, 2006). These results allow us to address how the cellular forces that promote organ-shaping movements are generated, and whether they are triggered by non-autonomous or autonomous cues. Unveiling these forces—and their nature—could allow us to understand how their modulation results in different shapes. For example, it is tempting to speculate that respiratory surfaces that develop into external or internal organs in different animals might ultimately depend on the generation of forces in opposite directions during organogenesis (Franch-Marro *et al*, 2006).

Size and shape

Different branches of the tracheal system have specific and distinct diameters and lengths. These features are stereotypical and it has been proposed that they are under the control of a genetic programme (Beitel & Krasnow, 2000). Recently, an attractive model has been proposed in which a chitinous filament inside the tracheal lumen defines tracheal tube size. In particular, it has been proposed that the fibrillar chitin matrix signals the tracheal cells to reorganize their cytoskeleton, in order to adjust their cell shape and create tubes of particular sizes (Tonning *et al*, 2005; Swanson & Beitel, 2006). However, all the mutants that alter tube size and/or the chitin filament also alter the correct organization of the tracheal cells and cuticle (see, for example, Behr *et al*, 2003; Hemphala *et al*, 2003; Paul *et al*, 2003; Llimargas *et al*, 2004; Wu *et al*, 2004; Araujo *et al*, 2005; Tonning *et al*, 2005; Luschnig *et al*, 2006; Wang *et al*, 2006), which makes it difficult to assess the direct role of the chitin filament in size determination. Irrespective of the final assessment of a role for the chitin filament, I would like to suggest that many features of tube size might not be under the independent control of a specific genetic programme, but instead that size might be a structural property of the organization of each specific branch. Accordingly, the size control of a particular tube would not be imposed on a branch, but would rather be a consequence of its cellular organization. For example, tube diameter could depend on whether two or more cells contribute to the branch circumference and could therefore be indirectly controlled by the programme regulating cell intercalation (Fig 3E). Similarly, tracheal cell shapes in the branches formed along the antero-posterior axis differ from those formed along the dorso-ventral axis; the former adopt an elongated shape, whereas the latter remain cuboidal (Llimargas & Casanova, 1999). These cell shapes are also related to the basic organization of the different tracheal branches, and are regulated by the specific signalling pathways responsible for migration in either axis. Therefore, once the basic organization of the distinct branches is set, the remaining process of lumen formation and the final thickness of the tracheal epithelium could be determinants of the final size of the tubes. In this regard, some features of size could be a consequence of the shaping mechanisms acting at the different steps of morphogenesis reviewed above.

Concluding remarks

The longstanding issue of shape generation remains a central topic in biology. Numerous studies in different systems have now allowed the specific events and mechanisms to be more accurately described. A more complete picture is emerging that allows one to highlight ideas that might be of general relevance. The examples reviewed here are derived from the study of *Drosophila* tracheal development; they reflect my viewpoint and are therefore, by definition, subjective. Furthermore, it must be noted that different systems can have specific traits; as I mentioned before, a peculiarity of *Drosophila* tracheal morphogenesis is that it takes place in the absence of cell proliferation, which is important in other morphogenetic processes. Similarly, interactions with neighbouring cells can contribute to varying extents to the development of different organs. However, regardless of such peculiarities, it will be interesting to see whether the notions reviewed here are relevant to other scenarios and represent general features of the generation of shape.

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